Performance characteristics to assess a receptor-ligand biosensor

William E. Lee
Shiliang Wang
Susan J. Rowsell
DRDC – Suffield Research Centre

Defence Research and Development Canada
Scientific Report
DRDC-RDDC-2017-R178
March 2018

IMPORTANT INFORMATIVE STATEMENTS

Disclaimer: Her Majesty the Queen in Right of Canada (Department of National Defence) makes no representations or warranties, express or implied, of any kind whatsoever, and assumes no liability for the accuracy, reliability, completeness, currency or usefulness of any information, product, process or material included in this document. Nothing in this document should be interpreted as an endorsement for the specific use of any tool, technique or process examined in it. Any reliance on, or use of, any information, product, process or material included in this document is at the sole risk of the person so using it or relying on it. Canada does not assume any liability in respect of any damages or losses arising out of or in connection with the use of, or reliance on, any information, product, process or material included in this document.

This document was reviewed for Controlled Goods by Defence Research and Development Canada (DRDC) using the Schedule to the *Defence Production Act*.

Endorsement statement: This publication has been peer-reviewed and published by the Editorial Office of Defence Research and Development Canada, an agency of the Department of National Defence of Canada. Inquiries can be sent to: Publications.DRDC-RDDC@drdc-rddc.gc.ca.

© Her Majesty the Queen in Right of Canada (Department of National Defence), 2018

© Sa Majesté la Reine en droit du Canada (Ministère de la Défense nationale), 2018

Abstract

Biosensors represent an important area of technology for biodetection, bioidentification, diagnostics, medical countermeasures, and human performance within the operations of the Canadian Armed Forces (CAF). The development of biological detection, identification, and monitoring (BioDIM) systems is a multi-step process that requires the assessment of candidate biosensor technologies at each step for further technology advancement. The CAF Directorate Chemical Biological Radiological Nuclear Defence (D CBRN D) sponsors research and development of biosensor technology within the DRDC CBRN Defence Program Inform Project. The present report contains an overview of the fundamental concepts associated with the predominant class of biosensors, those based on receptor-ligand interactions. Such biosensors comprise two distinct components, an electronic transducer device and molecular recognition elements (MREs) that together are able to detect particular chemical or biological analyte molecules. In a biosensor technology development project, normally the sponsor directs resources at the electronic transducer component plus hardware of the BioDIM system rather than the MREs. The focus of the report is on the performance characteristics of the transducer plus associated hardware, i.e., the areas where the sponsor would direct resources. This report outlines some performance characteristics of biosensors and provides analysis and guidance for assessing new biosensor technologies. The objective of the report is to provide CAF sponsors with tools and strategies for assessing biosensor development, hence to enhance the return on investment of DND resources.

Significance to defence and security

Biosensors based on receptor-ligand interactions are important technologies for biological detection, identification, and monitoring (BioDIM) systems. Biosensors can appear complicated to non-experts, i.e., staff personnel who are more connected to the end-user groups than to the technology itself. The description and analysis presented in this report deconstructs the problem to make the underlying issues in biosensor development more approachable to both the end-user and sponsors. The guidance contained herein will augment the success of current and future development. The technology readiness level of the biosensor herein is low, about two or three. Based on an increasing cost escalation per technology level, effective assessment at the low levels reduces risk at the more expensive higher levels. An effective technology assessment strategy is critical for return on investment of resources for technology advancement.

Résumé

Les biocapteurs représentent un domaine important de la technologie pour la biodétection, la bioidentification, le diagnostic, les contre-mesures médicales et le rendement humain dans le cadre des opérations des Forces armées canadiennes (FAC). La mise au point de systèmes de biodétection, bioidentification et biosurveillance (BioDIM) est un processus qui comporte de multiples étapes et qui nécessite l'évaluation des technologies de biocapteurs susceptibles d'être utilisées à chacune de ces étapes pour l'avancement technologique. La Direction – Défense chimique, biologique, radiologique et nucléaire, (DDCBRN) des FAC parraine la recherche et le développement de la technologie des biocapteurs au sein du projet INFORM dans le cadre du Programme de défense CBRN de RDDC. Le présent rapport contient un aperçu des concepts fondamentaux relatifs à la classe prédominante de biocapteurs, soit ceux basés sur les interactions récepteurs-ligands. Ces biocapteurs comportent deux composants distincts, un transducteur électronique et des éléments de reconnaissance moléculaire (ERM) qui, ensemble, permettent de détecter certaines molécules chimiques ou biologiques à analyser. Dans un projet de développement technologique de biocapteurs, le parrain affecte normalement les ressources au composant du transducteur électronique et au matériel du système de BioDIM, plutôt qu'aux ERM. Le présent rapport porte principalement sur les caractéristiques de rendement du transducteur et du matériel connexe, c.-à-d. les secteurs auxquels le parrain affecte normalement les ressources. Ce rapport présente les caractéristiques de rendement des biocapteurs et fournit une analyse, de même qu'un encadrement afin d'évaluer de nouvelles technologies de biocapteurs. L'objectif du rapport est de doter les parrains des FAC d'outils et de stratégies pour évaluer la mise au point des biocapteurs, et améliorer ainsi le rendement du capital investi dans les ressources du MDN.

Importance pour la défense et la sécurité

Les biocapteurs basés sur les interactions récepteur-ligand sont des technologies importantes des systèmes de biodétection, bioidentification et biosurveillance (BioDIM). Ils peuvent paraître complexes aux yeux des profanes, c.-à-d. le personnel fonctionnel qui est plus proche des groupes d'utilisateurs finaux que de la technologie. La description et l'analyse contenue dans le présent rapport permettent de décortiquer le problème dans le but de faciliter la compréhension des questions sous-jacentes à la mise au point de biocapteurs pour les utilisateurs finaux et les parrains. L'encadrement décrit ici facilitera la réussite des progrès actuels et futurs. De plus, le niveau de maturité technologique des biocapteurs examinés ici est faible : 2 ou 3 environ. Compte tenu de la hausse de l'indexation des coûts par niveau technologique, une évaluation efficace aux niveaux inférieurs permettra de réduire les risques plus coûteux aux niveaux supérieurs. Par ailleurs, une stratégie efficace d'évaluation des technologies est essentielle afin d'obtenir un rendement du capital investi dans les ressources pour l'avancement technologique.

Table of contents

Abstract . . . i
Significance to defence and security . . . i
Résumé . . . ii
Importance pour la défense et la sécurité . . . ii
Table of contents . . . iii
List of figures . . . iv
List of tables . . . vi
1 Introduction . . . 1
2 Methods and analyses . . . 3
3 Results and discussion . . . 4
3.1 Response to aqueous media . . . 4
3.2 Response to nonspecific binding . . . 5
3.3 Equilibrium receptor-ligand binding on an ideal biosensor . . . 6
3.4 Model system for receptor-ligand interactions . . . 9
3.5 Performance criteria of a biosensor . . . 13
3.5.1 Sensitivity, dynamic range, limit of detection, and limit of quantitation . . . 13
3.5.2 Specificity and selectivity . . . 18
3.5.3 Other characteristics and evaluation criteria . . . 19
4 Conclusions and comments . . . 20
References . . . 22
List of symbols/abbreviations/acronyms/initialisms . . . 24

List of figures

Figure 1: Layout of a notional nanowire (NW) sensor. Sketch (a) shows the transducer with receptor molecules (sulfur-dA_{20}) attached to the surface via sulfur-gold interactions. Sketch (b) shows ligand/analyte (dT_{20}) bound to receptor. . . . 2

Figure 2: The signal response to pH of sample solution of a notional sensor that measures current (I) through the transducer. The data collection for each run was approximately 60 sec as indicated by the double arrow. The abscissa (x-axis) is the run number; the ordinate (y-axis) is sensor signal (I) in arbitrary units. . . . 5

Figure 3: Equilibrium receptor-ligand binding. (a) A plot of normalized receptor-ligand binding (Y) versus ligand concentration according to Equation (8) for K_D = 1, 3, 10 nM. K_D is the point on the plot where Y = 0.5. Lower values of K_D correspond to stronger receptor-ligand binding. (b) A plot of Equation (8), normalized Y vs log ligand concentration. . . . 8

Figure 4: A notional real-time receptor-ligand binding experiment, for example, dA_{20}-dT_{20}. (a) The arrow indicates addition of ligand (e.g., dT_{20}) to the sensor. The signal increases upon receptor-ligand binding. (b) The arrow indicates addition of a negative control. In this example the negative control is non-thiolated dA_{20}, the same polymer as used as receptor but without the 3'-thiol moiety. The negative control would not be expected to bind to the sensor surface. No appreciable increase in signal is observed. (c) A "before-and-after" experiment for receptor-ligand binding. In this instance the ligand is added off-line; the kinetic signal is not measured. An equilibrium signal before and after addition of ligand is recorded. . . . 10

Figure 5: The main plot shows the dose response of an electrochemical impedance biosensor. The X-axis is the logarithm of concentration (moles/liter) of ligand (dT_{20}); the Y-axis is the change in the charge transfer resistance of the sensor. The sensor monitored the binding of ligand to the receptor (dA_{20}) as shown in Figure 1. The main plot shows a nonlinear regression of data to a receptor binding model Y=Ymax/(1 + 10^(logK_D - X)*Hill index) where X is the logarithm of concentration (moles/litre) and Ymax (9.26), log K_D (-8.4) and Hill index (0.38) [12] were determined from the data fit (R^2=0.998). Error bars are not shown. The inset shows the same data as a linear regression of four data points at log concentration -10 to -7 (R^2=0.99). The change charge transfer resistance (y-axis) is shown in ohms. The dashed line is the signal arising from addition of non-complementary target (i.e., negative control), dA_{20} [7].. . . . 11

Figure 6: An extended data set for the experiment depicted by Figure 5. (a) A plot of signal (ΔR_{ct} kΩ) versus logarithmic molar concentration dT_{20} (ligand). (b) same data plotted versus linear concentration. (c) an expanded view of the low concentration regime plot in linear concentration. . . . 15

Figure 7: The low concentration regime as shown in Figure 6c with a blank sample ($[dT_{20}] = 0$) included in the data set. The calibration curve as determined by linear regression was $y = (1.02\ k\Omega/nM) \cdot x + (1.726 \pm 0.0125)\ k\Omega$ The horizontal dashed line is Y-intercept + 3 SD, i.e., $Y_{LOD} = 1.726 \pm 3 \times (0.0125)$. The vertical dashed line is the LOD concentration; the interpolated value for X_{LOD} is about 0.042 nM. 16

List of tables

Table 1: Distribution of scenario evaluation weightings. 19

1 Introduction

Biosensors will have many applications in the future for biodetection, bioidentification, diagnostics, medical countermeasures, and human performance in the Canadian Armed Forces (CAF). The development of biological detection, identification, and monitoring (BioDIM) systems requires the assessment of candidate biosensor technologies for potential use in the these systems. This report outlines some performance characteristics of biosensors and provides analysis and guidance for assessing new biosensor technologies. The main example used herein is a sensor technology, nanowire field effect transistor (NWFET), which is being sponsored by the CAF Directorate Chemical Biological Radiological Nuclear Defence (D CBRN D) within the DRDC CBRN Defence Program Inform Project. The present report is a follow-up to an earlier communication entitled "Model analyte systems to assess nanowire biosensors" [1]. The focus of the latter was on reagents and wetware that could be used to assess the NWFET sensor. The present report addresses the performance characteristics of the sensor hardware itself. Together the documents comprise a complementary approach to evaluating sensor technology; specifically for NWFET, but applicable to other sensors in current and future development.

This report is an overview of biosensors employing receptor-ligand binding. Such biosensors comprise two distinct components, an electronic transducer device and molecular recognition elements (MREs) that together are able to detect particular chemical or biological analyte molecules [2, 3, 4]. The transducer device converts a signal in one form of energy to another form which can be measured. In context of the NWFET transducer, perturbations or changes in the electric field at the nanowire surface are converted to changes in output current. A functioning biosensor platform consists of the transducer device with immobilized MREs on the surface plus electronic interface to collect and record the output signal. MREs are usually drawn from bioactive molecules such as antibodies, nucleic acid probes, aptamers or enzymes. Every particular MRE has a molecular target (or analyte) to which it binds by means of receptor-ligand interactions. For electronic and electrochemical sensors, the receptor-ligand interactions on the sensor surface release chemical energy which then becomes the input signal for the transducer. Since the analyte materials for biosensors are usually larger sized chemical or biological molecules (neither gases nor vapors), the binding interactions on the sensor surface take place within a liquid (aqueous) medium. This required liquid carrier medium plus the MREs are referred to as the wetware.

In order for a biosensor to function, both hardware and wetware must function effectively. For development of the transducer component of the sensor it is critical to employ easy-to-use and simple MRE/target (i.e., receptor/ligand) pairs in the wetware. Model analyte systems to assess biosensors have been described previously [1]. The objective of the present report is to provide context to the performance characteristics associated with the hardware component of the biosensor. A typical layout for a biosensor is given in Figure 1 showing a NWFET transducer and complementary nucleic acids as a receptor-ligand pair. Development of an indium arsenide NWFET has been reported previously [5, 6].

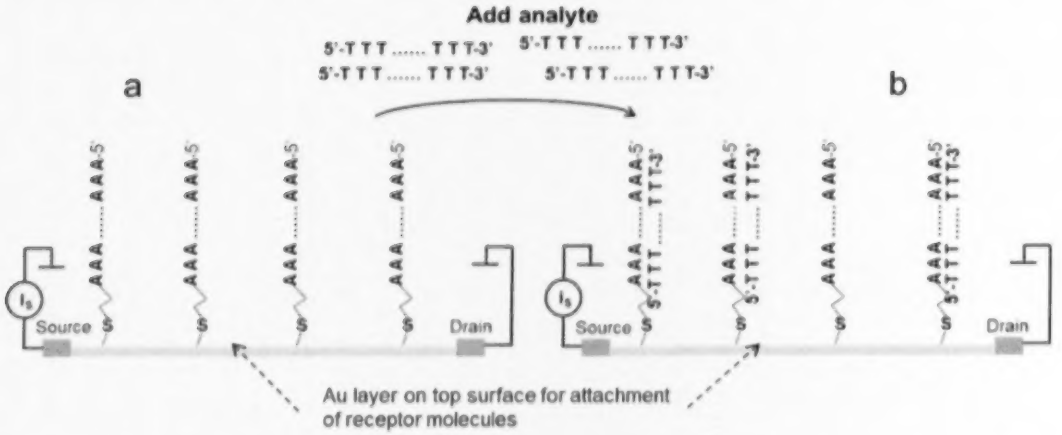

Figure 1: *Layout of a notional nanowire (NW) sensor. Sketch (a) shows the transducer with receptor molecules (sulfur-dA_{20}) attached to the surface via sulfur-gold interactions. Sketch (b) shows ligand/analyte (dT_{20}) bound to receptor.*

2 Methods and analyses

The analytical process herein for assessing the performance characteristics of a biosensor is applicable to most biosensors based on receptor-ligand interactions. The present example is a NWFET sensor as depicted in Figure 1. The receptor-ligand molecules for this example are single-stranded DNA oligomers. The receptor is thiolated probe 5'-AAA AAA AAA AAA AAA AAA AA/3ThioMC3-D/-3' (dA_{20}-thiol); the ligand is 5'-TTT TTT TTT TTT TTT TTT TT-3' (dT_{20}); the non-complementary negative control is 5'-AAA AAA AAA AAA AAA AAA AA-3' (dA_{20}).

The modifications to the transducer surface shown in Figure 1 represent a notional (or hypothetical) fabrication of a receptor-ligand sensor. Although notional, the surface structures are based on standard methods and are similar to previous work [7, 8]. In this example, the transducer surface (nanowire) is modified with a layer of gold or is decorated with gold nanoparticles. The nanowire is further modified by immersing in a solution of receptor molecule, dA_{20}-thiol, to produce the sensor surface (Figure 1a). The use of thiol molecules (sulfhydryl-containing molecules) is an expedient method for preparing sensor surfaces whereby the thiol molecules create a self-assembled monolayer on the gold surface [7]. The ligand molecule, dT_{20}, when added to the sensor surface spontaneously hybridizes to the receptor as indicated by Figure 1b. The formation of receptor-ligand pairs generates the signal within the sensor.

Figures 2 and 4 represent notional (hypothetical) experiments to measure basic responses of a sensor. Figure 3 represents original simulations for a simple 1-receptor/1-ligand model as described in the results and discussion section. Electrochemical impedance spectroscopy (EIS) data (Figure 5) was taken from previous work by the authors [7] as an example of receptor-ligand binding. In this example the same receptor-ligand pair (dA_{20}-thiol and dT_{20}) as shown in Figure 1 was used. Figures 6 and 7 represent a new and original re-analysis of the data for the purpose herein of assessing performance characteristics. All simulations and nonlinear regression analyses were performed on GraphPad Prism 6.0 software (GraphPad Software Inc. San Diego, CA).

3 Results and discussion

The scope of sensor development and assessment is broad and contains many layers. The review and analysis contained in this report are directed at general characteristics of biosensor performance rather than at optimization of design, manufacture or assay formulations. These general characteristics of performance include the following.

Basic performance: Compatibility of the biosensor with the concept of use. The biosensor should be able to function in the presence of aqueous media, bioassay reagents and non-analyte macromolecules such as proteins, polysaccharides, nucleic acids, etc.

Specific performance: The biosensor should possess molecular recognition for compound(s) or molecules of interest. At a basic level the biosensor should be able to discriminate between analyte and non-analyte material.

Analytical performance: The biosensor should display a measureable sensitivity to analyte. In other words, a plot of signal versus concentration will show a dose response, usually a positive slope (ΔSignal/ΔConcentration) when analyte is presented to it. The operator of biosensor should be able to determine a limit of detection for the analyte. A biosensor that contains receptors (and ligands) should demonstrate the fundamentals of receptor-ligand binding. Experimental results from the biosensor should be reproducible.

3.1 Response to aqueous media

A constraint on sensor systems designed to detect biomolecules is the requirement to function in aqueous media. Thus the response of the sensor to aqueous phase medium itself is a key characteristic. The main factors in bulk aqueous medium are pH (hydrogen ion concentration) and electrolyte (salt) concentration. A simple and fundamental experiment to characterize the sensor in an aqueous medium would be the response to change in pH. A notional series of sensor experiments using standard buffers ranging from basic (high) pH to acidic (low) pH is shown in Figure 2. In this example each sample is monitored in real time for about 60 seconds. The experiment shows an increase in signal (current) with decrease in pH (decreased pH represents increased hydrogen ion concentration). The sensor output remains constant over the course of each run, demonstrating the stability of the device. The noise fluctuation on the individual runs is relatively constant as well, about 0.5–0.75 a.u. Thus as the signal increased, so does the signal-to-noise ratio (S/N) from about S/N = 5 at pH 9 to S/N = 60 at pH 4.

A real-world experiment of a sensor under development (e.g., NWFET) might show different response with respect to pH of the aqueous medium but would be still responsive and reproducible to the change in the aqueous medium. The magnitude of the signal change would provide knowledge of the sensitivity of the sensor to pH. If the sensor is highly sensitive to pH, then pH control becomes an important issue for analytical experiments. The aqueous medium in bioassays is usually phosphate buffered saline, 10 mM sodium phosphate pH 7.2, 150 mM sodium chloride. Overall this medium is approximately 1% electrolyte, primarily sodium chloride. Thus the response of the sensor to electrolyte concentration and ionic strength is a useful characteristic to assess, although this example is not included herein. Since the sensor has two

components, transducer and MREs, the above response to changes in aqueous medium could be performed on the transducer itself, i.e., bare transducer and on the functioning sensor (transducer plus MRE). Both experiments would provide useful information.

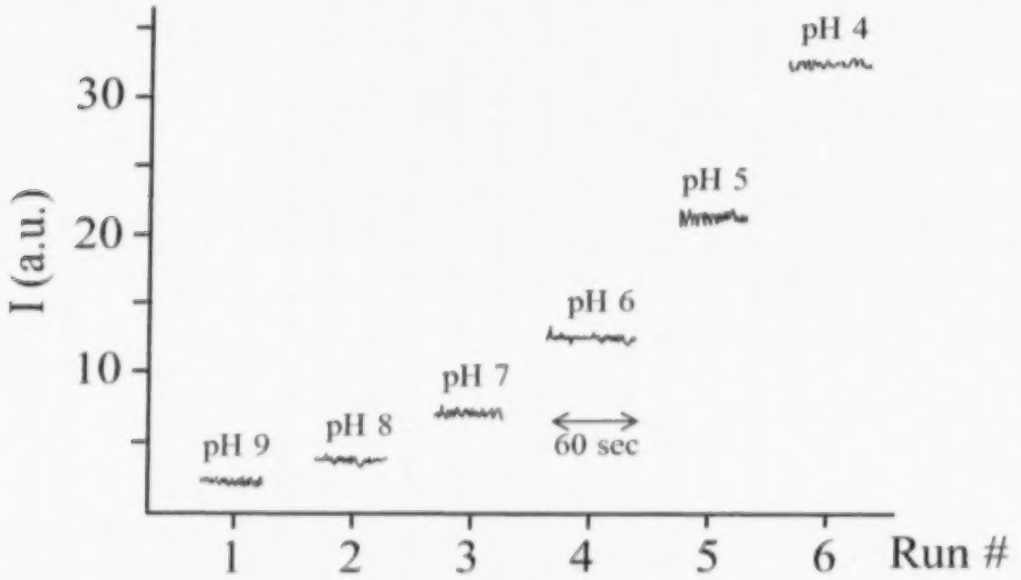

Figure 2: *The signal response to pH of sample solution of a notional sensor that measures current (I) through the transducer. The data collection for each run was approximately 60 sec as indicated by the double arrow. The abscissa (x-axis) is the run number; the ordinate (y-axis) is sensor signal (I) in arbitrary units (a.u.).*

3.2 Response to nonspecific binding

The interaction of the sensor receptor with ligand (analyte) is referred to as specific binding. Other interactions of biomolecules in the aqueous sample with the sensor system are referred to as nonspecific binding (NSB). There are several ways whereby NSB can interfere with sensor performance. It is possible for ligand (analyte) to stick to non-receptor sites on the container walls or adsorb to a sample matrix if such were present. This type of NSB would reduce the output signal and hence reduce the concentration of ligand (analyte), potentially yielding false negative results. However it can be minimized by saturating these unoccupied non-receptor binding sites with blocking agent that, itself, does not take part in the specific receptor-ligand interaction. Common blocking agents are proteins (albumin, casein, gelatin, whole sera) and polymers (such as polyethylene glycol, polyvinyl alcohol) [8].

Another example of NSB is non-ligand materials (impurities, sample matrix components, reagents) adhering to the sensor receptor which would increase the signal, potentially leading to false positive results. This latter type can be reduced through the use of detergents (Tween-20, Triton X-100) [9] in the assay wetware and commercial blocking agents [10]. NSB also occurs when non-ligand material adheres to non-receptor sites on the transducer surface. Usually any

material that sticks to the transducer, either through specific receptor-ligand binding or at non-receptor sites, registers as signal. Thus, the occurrence of NSB adds false signal to the assay. This situation can be minimized by immersing the sensor surface (transducer plus receptor) in a solution of blocking agent for a specified period of time in order to fill all these non-receptor sites, after which blocking solution is rinsed away. Any blocking agent that adheres to the transducer during immersion contributes to the background signal (the baseline) rather than to the analyte signal. Although nonspecific binding is an important issue to assess in assay development for biosensors, it is not a hardware problem per se. It is wetware problem that is to be addressed at the assay development stage. However developers should be mindful of the NSB issues throughout.

3.3 Equilibrium receptor-ligand binding on an ideal biosensor

The biosensor design described in this report incorporates receptor-ligand interactions for detection of analyte. This interaction can be written as

$$R + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} RL \qquad (1)$$

where R is the receptor immobilized on the surface of the sensor, L is ligand and RL is receptor-ligand complex also immobilized on the sensor's surface; k_a and k_d are the reaction rate constants in the forward direction (association), and in the reverse direction (dissociation), respectively. Concentrations of reactants are denoted by square brackets, e.g., ligand concentration is written as [L].

The rate of formation (association) of RL is the forward reaction

$$d[RL]/dt = k_a [R] \cdot [L] \qquad (2)$$

The rate of disappearance of RL (dissociation) is the reverse reaction

$$d[RL]/dt = k_d [RL] \qquad (3)$$

At equilibrium the net change in [RL] is zero, i.e., the forward and reverse reactions are equal. Thus,

$$k_a [R] \cdot [L] = k_d [RL]$$

$$k_d / k_a = [R] \cdot [L] / [RL] \quad \text{or}$$

$$K_D = [R] \cdot [L] / [RL] \; ; \quad (\text{where } k_d / k_a = K_D) \qquad (4)$$

K_D is the equilibrium dissociation constant and has dimensions of concentration, e.g., moles per liter. Usually in chemical (or biochemical) kinetics, rate constants are denoted by lower case, e.g., k, and equilibrium constants by upper case, K.

In a biosensor, the formation of occupied receptor sites, [RL], initiates the signal in the sensor; unoccupied sites, [R], do not contribute to signal. The total number of receptor sites on the sensor is the sum of occupied and non-occupied sites and can be expressed as

$$[R]_{total} = [R] + [RL]$$

The fraction of occupied receptor sites, f_R, can be written as

$$f_R = [RL] / [R]_{total} = [RL] / ([R] + [RL]) \quad (5)$$

Equation (4) can be rearranged as $[RL] = [R] \cdot [L] / K_D$

and substituted for [RL] in Equation (5) above so that

$$f_R = 1 / (1 + (K_D/[L])) \quad (6)$$

where [L] is free ligand [11]. In a receptor-ligand experiment described by Equation (6), the variable [L], which is required to solve the equation, is often difficult to measure. However for saturation binding the total ligand in the experiment is usually much higher than the total receptor. Thus, free ligand [L] is effectively equal to total ligand, $[L]_T$ (a known value) and Equation (6) can be approximated as

$$f_R = 1 / (1 + (K_D/[L]_T)) \quad (7)$$

Equation (7) assumes a single class of receptor, a single class of ligand and there is no cooperation or anti-cooperation between binding sites.

In the biosensor experiment, the occupied binding sites (the RL complexes) generate the signal proportional to the amount of RL present. Thus the signal, Y, can be expressed as

$$Y/Y_{max} = 1 / (1 + (K_D/[L]_T)) \quad (8)$$

where Y is the signal for a given concentration of $[L]_T$ and Y_{max} is the maximum (saturation) signal at high $[L]_T$. Equation (8) further assumes that the amount of signal generated per bound ligand molecules is independent of the number of occupied binding sites. In other words, each additional receptor-ligand interaction contributes the same amount of signal to the sensor. In the discussion, equations and plots of signal versus ligand concentration below, the approximation of free ligand to total ligand has been used.

From inspection the limits of Y/Y_{max} can be determined as,

$[L] \rightarrow 0, \quad Y/Y_{max} \rightarrow 0,$

$[L] \rightarrow \infty$ (high concentration limit), $\quad Y/Y_{max} \rightarrow 1.$

When $[L] = K_D$, $\quad Y/Y_{max} = 0.5$

A plot of Y/Y_{max} versus [L] in Equation (8) gives a rectangular hyperbola with horizontal asymptote value of 1.0 [12]. The function in Equation (8) has a single variable, $[L]_T$ and a single parameter, K_D. A plot of Equation (8) is shown in Figure 3a. The strength of the receptor-ligand binding is reflected through K_D; the stronger the binding, the lower the dissociation constant K_D. Thus for stronger receptor-ligand binding pairs, the midpoint of the plot ($Y/Y_{max} = 0.5$) shifts to lower values of [L] as shown in Figure 3a. The same binding as defined by Equation (8) is shown

in Figure 3b where Y/Y_{max} is plotted versus log [L], however in this case the plot is a sigmoid curve. The former (Figure 3a) shows linear concentration of ligand and the latter (Figure 3b) logarithmic. In Figure 3b, the change in K_D, from 10 nM to 1 nM results in a shift of the inflection point of the curve towards the left hand side (i.e., towards Y-axis). The overall shape remains the same. It is important to remember that Figures 3a and 3b display the same data and same binding processes; they are the same function with linear or logarithmic x-axis.

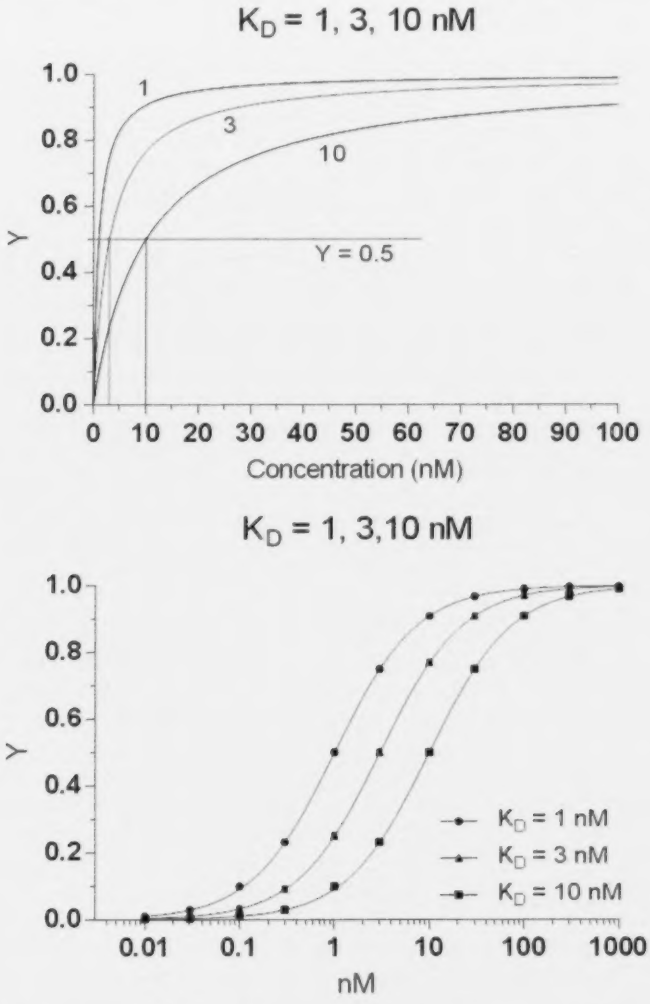

Figure 3: *Equilibrium receptor-ligand binding. (a) A plot of normalized receptor-ligand binding (Y) versus ligand concentration according to Equation (8) for K_D = 1, 3, 10 nM. K_D is the point on the plot where Y = 0.5. Lower values of K_D correspond to stronger receptor-ligand binding. (b) A plot of Equation (8), normalized Y vs log ligand concentration.*

3.4 Model system for receptor-ligand interactions

A simple and effective model receptor-ligand pair for assessing a biosensor is nucleic acids polydeoxyadenine (dA_{20}) and polydeoxythymine (dT_{20}). Adenine and thymine are complementary nucleotides in DNA and the polymers readily hybridize (i.e., form pairs) as shown in Figure 1. This nucleic acid model requires immobilizing the receptor (in this case dA_{20}-thiol) to the sensor surface. Figure 1 shows immobilization via gold-sulfur bonding. This method is convenient and has been used by the authors previously [7]. A kinetic (time course) hybridization experiment would resemble the traces shown in Figure 4. The arrow indicates the addition of ligand (i.e., the analyte) to the sample compartment. The rise in signal corresponds to the formation of the RL complex on the surface. Unbound ligand that is in the aqueous medium does not contribute to signal. The rise in the signal is dependent upon the diffusion rate of the ligand to the sensor surface and the time required to form RL complexes. For Figure 4a, the rise time is about 15 s, comparable to other biosensor systems that are diffusion dependent [13]. After about 100 s the signal reaches an equilibrium level. Figure 4b represents a test molecule not expected to bind to the sensor (i.e., a negative control), in this example dA_{20} (the non-thiol nucleic acid polymer, i.e., without the gold-adhering sulfur moiety). The arrow on the figure indicates the addition of the negative control to the sensor; no signal above the background noise is observed. In terms of sensor characterization Figure 4 is the first level. It confirms that the system functions as a sensor.

Figure 4a	ligand added	→	signal returned
Figure 4b	negative control added	→	no signal returned

The law of mass described by Equations (1)–(4) above applies to the equilibrium state of the receptor-ligand reaction. If the experiment shown in Figure 4a was repeated over a range of concentrations, a family of signal-versus-time traces would be obtained wherein a greater amount of analyte added would lead to greater equilibrium-state signals [13]. Some biosensor systems are not conducive to easily obtaining kinetic (time course) data as shown in Figures 4a and 4b. Rather, equilibrium data from before and after addition of analyte, resembling Figure 4c, are obtained. Figure 4c data are adequate for biosensor detection purposes. The critical information from Figure 4 is the presence of the steady baseline signal before analyte is added and the increase in signal at equilibrium after addition of analyte sample.

Figure 5 shows an equilibrium dose response of an electrochemical impedance biosensor based on previous work by the authors [7]. The receptor is dA_{20}-thiol, immobilized on a gold electrode; the ligand is dT_{20}. The interaction of dA_{20} and dT_{20} is analogous to Figure 1. The signal (kilo-ohms) is plotted versus the logarithm of the ligand concentration [L]. The inset shows a region of the dose response curve (0.1 to 100 nM) that can be fitted to a linear regression ($R^2 = 0.990$). In the main plot however the whole data set can be non-linearly fitted with a version of Equation (8) ($K_D = 4$ nM, $R^2 = 0.998$). The purpose of the comparison is to show that experimental data can appear linear ($R^2 \geq 0.9$) even though there is no underlying linearity in the process. Figure 5 (inset) is typical of a biosensor development experiment. The experimenter collects data "where the action is," that is the region of the process that shows the greatest response, and then reports a linear response over three orders of magnitude (0.1-100 nM dT_{20}). There is nothing incorrect with the assertion of "linear response over three orders of magnitude" but it may leave the impression that receptor binding was an inherent linear process.

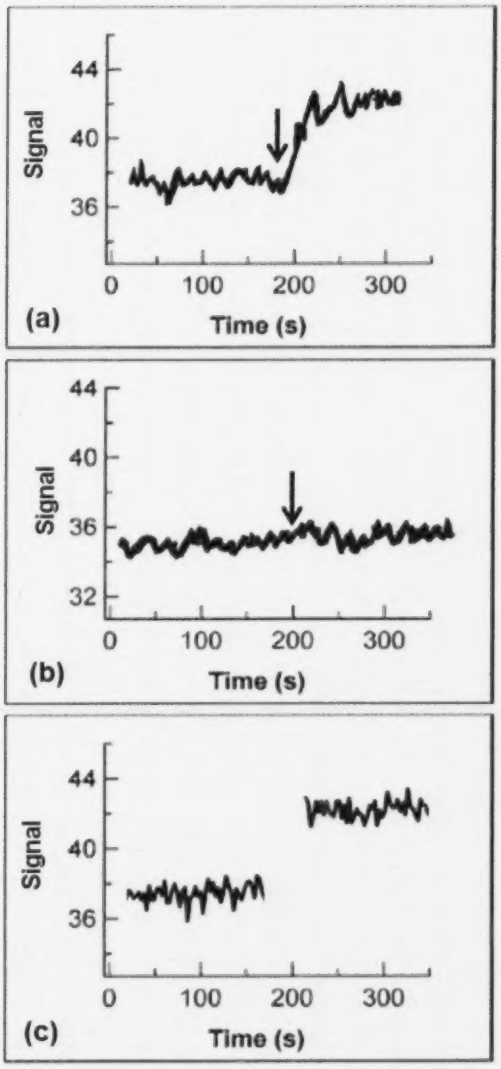

Figure 4: *A notional real-time receptor-ligand binding experiment, for example, dA_{20}-dT_{20}. (a) The arrow indicates addition of ligand (e.g., dT_{20}) to the sensor. The signal increases upon receptor-ligand binding. (b) The arrow indicates addition of a negative control. In this example the negative control is non-thiolated dA_{20}, the same polymer as used as receptor but without the 3'-thiol moiety. The negative control would not be expected to bind to the sensor surface. No appreciable increase in signal is observed. (c) A "before-and-after" experiment for receptor-ligand binding. In this instance the ligand is added off-line; the kinetic signal is not measured. An equilibrium signal before and after addition of ligand is recorded.*

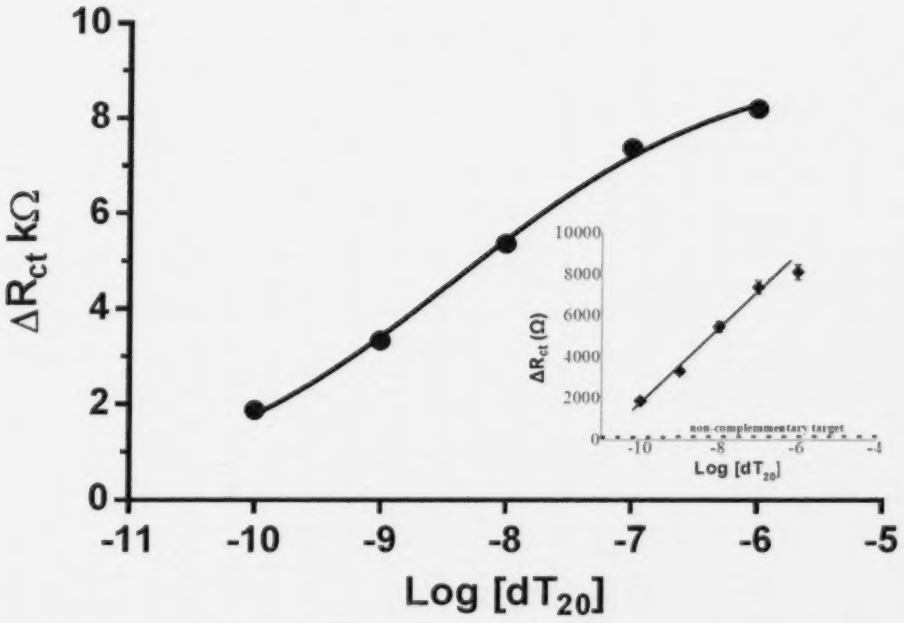

Figure 5: *The main plot shows the dose response of an electrochemical impedance biosensor. The X-axis is the logarithm of concentration (moles/liter) of ligand (dT_{20}); the Y-axis is the change in the charge transfer resistance of the sensor. The sensor monitored the binding of ligand to the receptor (dA_{20}) as shown in Figure 1. The main plot shows a nonlinear regression of data to a receptor binding model Y=Ymax/(1 + 10^(logK_D - X)*Hill index) where X is the logarithm of concentration (moles/litre) and Ymax (9.26), log K_D (-8.4) and Hill index (0.38) [12] were determined from the data fit (R^2=0.998). Error bars are not shown. The inset shows the same data as a linear regression of four data points at log concentration -10 to -7 (R^2=0.99). The change charge transfer resistance (y-axis) is shown in ohms. The dashed line is the signal arising from addition of non-complementary target (i.e., negative control), dA_{20} [7].*

Figure 6 shows a more complete data set for the experiment than is depicted in Figure 5. The dose response is extended at both low and high concentration regions. At low and high concentrations the curve approaches asymptotic values (Figure 6a). The extended data set resembles the sigmoid (semi-log) plot that would be expected from equilibrium saturation binding given by Equation (8) and shown in Figure 3b. The semi-log plot is useful because it displays the broad concentration range required for a complete saturation binding experiment. The same experimental data set when plotted versus linear concentration (Figure 6b) shows regions of linearity too, although at different (lower) concentrations and has similar characteristics to the family of plots in Figure 3a. In Figure 6b the data at concentrations above 10 nM were truncated in order to allow inspection of the lower concentration data. Even in this view the data points below 0.5 nM are too compressed to be examined. Figure 6c gives an expanded view of the low concentration region. In this view the data is linear in appearance.

Returning to Equation (8)

$$Y/Y_{max} = 1 / (1 + (K_D/[L]_T)) \quad (8)$$

At the low concentration regime when $[L] << K_D$, i.e., 2 orders of magnitude lower,

$$K_D/[L] \approx 50\text{-}100$$

and much larger than 1.0. The denominator of Equation (8) can be re-written

$$(1 + K_D/[L]) \approx K_D/[L] \quad (9)$$

However the assumption made above for Equations (7) and (8) is that free ligand is in excess. At low concentration of ligand, i.e., a regime of ligand depletion, this assumption is no longer valid. K_D is replaced by $K_{D\ app}$, the apparent equilibrium dissociation constant at low [L]. $K_{D\ app}$ is still large with respect to [L] so Equation (9) becomes

$$(1 + K_D/[L]) \approx K_{D\ app}/[L] \quad (10)$$

This condition of ligand depletion has been observed and discussed in detail elsewhere [14]. Substituting Equation (10) in Equation (8) gives

$$Y/Y_{max} \approx 1 / (K_{D\ app} / [L]) = [L] / K_{D\ app} \quad (11)$$

Equation (11) predicts a linear response of [L] at low concentration with slope of $1/K_{D\ app}$, and is shown as such in Figure 6c. The data in Figure 6c yielded $K_{D\ app}$ value of about 1 nM. The nonlinear curve fit for Figure 5 gave K_D value in the range of 4 nM. The lower value of $K_{D\ app}$ observed here is consistent with the previous observations [14]. The discrepancy between K_D values at low and high concentrations does not create a problem for sensor development. The purpose of the biosensor evaluation experiment is not to determine the dissociation constant of the receptor-ligand. It is to determine whether the biosensor response has analytical potential for a biodetection platform. The important criteria are detection limits, ease of use and reproducibility of the sensor experiments. An example of reproducibility would entail a new batch of sensors (transducers plus MREs) to provide similar results to the previous batch. Also the experiments should be reproducible by different sets of hands and in different laboratories.

An overall awareness of the issues of receptor ligand binding is useful for sensor development. It provides internal verification of the quality of the work. It also provides the project managers with investigative tools for evaluating third party research and development. Experiments with biosensors should have basic features:

- a general hyperbolic shape when signal is plotted versus a wide linear concentration range of ligand (2–3 orders of magnitude) as in Figures 3a and 6b;
- a sigmoid shape when signal is plotted versus log of concentration (5–6 orders of magnitude) as in Figures 3b and 6a; and
- a linear plot when signal is plotted versus linear low concentration, as in Figure 6c.

If these basic features are not apparent then there are likely problems with the experiments or the technology that should be addressed. In the EIS example used herein (Figure 5 inset) there were insufficient data points at the extremities of concentration for a full analysis. However the limited data were useful to establish the methods required to create an electrochemical impedance biosensor and to demonstrate the sensitivity and reproducibility of the technology.

3.5 Performance criteria of a biosensor

3.5.1 Sensitivity, dynamic range, limit of detection, and limit of quantitation

Many of the terms used in bioanalysis and sensors applications have similar connotations but different meanings, namely, sensitivity, dynamic range, selectivity, limit of detection, and limit of quantitation. These terms are often misused. The words denote properties of the sensor. Some of the properties are inherent to the transducer itself and some inherent to MREs and wetware; other properties are dependent on transducers, MREs and wetware.

For example, the signal generated in a sensor via receptor-ligand interaction is mostly a property of the transducer. Each type of transducer (e.g., NWFET versus EIS) will respond to a different type of perturbation or stimulus resulting from the ligand adhering to the sensor surface. For NWFET, the perturbation is in the electric field on the surface of the nanowire; for EIS, it is the charge transfer resistance through the sensor surface. In order for the ligand molecules to adhere to the surface, receptor molecules are required but the signal itself is a property of the transducer. Conversely, in a liquid sample presented to the sensor, whether a biological molecule is a ligand or non-ligand is a property of the receptor (molecular recognition element). The sensor will only generate signal if the molecule sticks to the surface.

The sensitivity of a sensor is defined by the slope of the dose-response curve [15]. The sensitivity is dependent upon both the response of the transducer to a captured ligand molecule and to the ability of the receptor to capture. In other words it is the change in signal response (ΔR) per unit amount of analyte, usually concentration (ΔC). This is best seen on Figure 3a where sensitivity is $\Delta R/\Delta C$. Normally the sensitivity of the sensor continuously decreases with increasing concentration of ligand. For instance, at saturation (high concentration limit) the sensitivity decreases to zero. The concentration over which the sensor shows appreciable signal response is referred to as the dynamic range. For a single receptor-single ligand model, the dynamic range is best shown in Figure 3b. The region of concentration for the dynamic range is determined by K_D, the inflection point of the plot. According to Equation (8), at ligand concentration, [L] equal to 1 log unit less than K_D (i.e., $K_D / [L] = 10$),

$$Y/Y_{max} = 1 / (1 + 10) = 0.0909$$

at 1 log unit greater than K_D (i.e., $K_D / [L] = 0.1$

$$Y/Y_{max} = 1 / (1 + 0.1) = 0.9091$$

For the family of curves in Figure 3b the breadth of the dynamic range is the same; only the position of the inflection point changes. On the semilog plot the slope of the line from 1 log unit below K_D ($Y/Y_{max} = 0.0909$) to 1 log unit above ($Y/Y_{max} = 0.9091$) is

$$\text{Log slope} = (0.9091 - 0.0909) / 2 = 0.409 \text{ per log unit.}$$

The slope of this line is independent of the value of K_D and independent of receptor-ligand pair, provided the pair conforms to Equation (8). In practice there are factors which broaden the transitions (as indicated above, incremental decrease of signal per additional ligand molecule, or anti-cooperative interactions between receptor sites). The data in Figure 5 show a broader dynamic range than predicted by Equation (8). The comparable slope is on Figure 5 is 0.22 per log unit. Therefore any factor that would broaden the dynamic range would also decrease the sensitivity of the sensor.

In principle the greater the sensitivity (i.e., slope of the signal versus concentration curve) the greater the resolving power of the sensor to distinguish differences between samples, especially between samples of low concentration of analyte and the blank sample. The concept of sensitivity should not be confused with limit of detection (LOD). LOD includes consideration of the experimental error and noise in the analysis, in addition to slope. The value of K_D has a critical role in determining LOD of a sensor. The lower the K_D value, the lower the concentration of the onset of signal as shown by Figure 3a. The conventional definition of LOD is the point on the calibration curve equal to the mean blank sample plus three standard deviations (SD), a confidence level of 99% [15]. For example, Figure 7 shows a low concentration calibration curve with a blank sample (0 nM). LOD was determined to be about 0.043 nM by interpolation (see caption).

Another approach for determining LOD is Figure 6c, which shows the low concentration calibration curve without the blank sample. In this case, even without the blank sample, the LOD can be determined from the linear regression of the data ($y = mx + b$) which yields m (slope) = 1.02 kΩ/nM and b (y-intercept of the linear regression) = 1.726 ± 0.0125 kΩ.

The LOD value can be calculated from the linear regression data

$$Y_{LOD} = m \cdot X_{LOD} + b \tag{12}$$

(m is slope and b is y-intercept). Left hand side of Equation (12) can be written as it is defined above (the LOD is equal to the signal of the blank signal plus three SDs of the blank signal)

$$Y_{LOD} = b + 3 \cdot SD \tag{13}$$

and is equal to the right hand side of Equation (12).

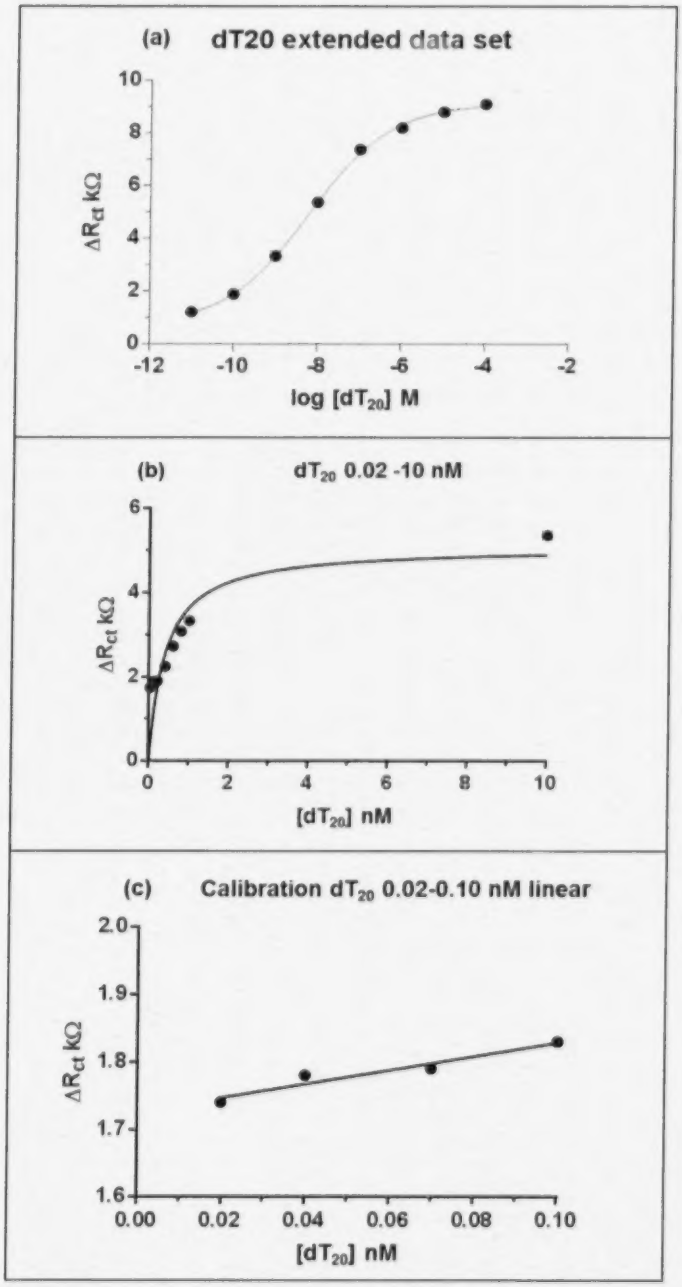

Figure 6: *An extended data set for the experiment depicted by Figure 5. (a) A plot of signal (ΔR_{ct} kΩ) versus logarithmic molar concentration dT_{20} (ligand). (b) same data plotted versus linear concentration. (c) an expanded view of the low concentration regime plot in linear concentration.*

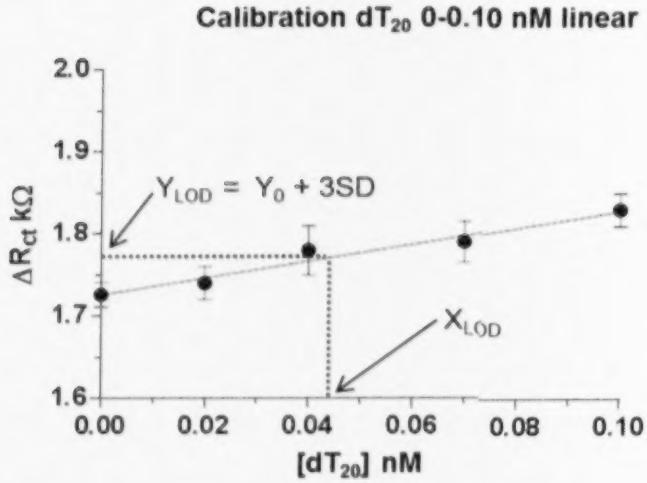

Figure 7: *The low concentration regime as shown in Figure 6c with a blank sample ($[dT_{20}] = 0$) included in the data set. The calibration curve as determined by linear regression was*
$y = (1.02\ k\Omega/nM) \cdot x + (1.726 \pm 0.0125)\ k\Omega$
The horizontal dashed line is Y-intercept + 3 SD, i.e., $Y_{LOD} = 1.726 \pm 3\ x\ (0.0125)$.
The vertical dashed line is the LOD concentration;
the interpolated value for X_{LOD} is about 0.042 nM.

Thus combining Equations (12) and (13)

$$b + 3 \cdot SD = m \cdot X_{LOD} + b$$

Simplify

$$3 \cdot SD = m \cdot X_{LOD}$$

$$X_{LOD} = 3 \cdot SD/m \qquad (14)$$

From the linear regression of Figure 6c,

$$X_{LOD} = (3 \times 0.0125\ k\Omega) / (1.02\ k\Omega/nM) = 0.037\ nM$$

Both methods yield similar estimates of LOD. Equation (14) is a very useful relationship, namely, the LOD of an assay is 3 x SD of the y-intercept divided by slope of calibration curve. In essence, Equation (14) states that LOD is directly proportional to the standard deviation of the data points in the low concentration portion of the calibration curve and inversely proportional to the slope.

Based on a normal distribution of measurement data, a sample having a signal at the LOD has a 99+% confidence level of being a positive sample. The likelihood of a false positive (being a blank sample) is about 1%. In other words, a true blank sample has a 1% probability of being scored at the LOD cut-off or higher (positive). Thus a false positive is unlikely. For the same sample the probability of false negative is 50%. In other words if the true value of the sample is at the LOD cutoff, the assay has a 50% probability of rejecting the sample as being below the

cut-off [16, 17, 18]. To this day there is still confusion and debate over the meaning of LOD with respect to positive/negative vs true/false. For this reason the term "limit of quantification" (LOQ) is taken to be the blank plus 10 SD. At this level the probability of false negative (or positive) is minimal. If the region of the calibration curve spanning LOD and LOQ is linear then LOD defines LOQ.

$$LOQ = (10/3) \times LOD \quad (15)$$

In determining LOD and LOQ there is a hidden assumption, namely, the standard deviation on the blank is equal to the standard deviation on the samples in the region of LOD and LOQ. It is always good practice to calculate LOD by interpolation and by Equation (14) if the data permits. The LOD and LOQ values, determined in an ideal laboratory assessment, should not be considered real-world values for deployment purposes.

A less rigorous but more real-world approach to determination of LOD was used by US Army Edgewood Biosensor Test Bed [19, 20]. In this approach an initial estimate of LOD (based on manufacturer claims) was made. If two consecutive initial samples yield signals that are above the background sample (i.e., yield positive signals) then dilute the sample by 10 and repeat until fewer than two consecutive positive results are returned. The lowest concentration to give two consecutive positive results is the "Test-Bed LOD." If samples at the estimated concentration return fewer than two consecutive positive signals, then serially increase the concentration by 10 times and repeat. Again the lowest concentration that returns two consecutive positive signals is the Test-Bed LOD. This experimental approach would be useful to the CAF since it allows comparison to US Army performance assessments and field trials.

For the sensor developer LOD should be considered a benchmark. Simply put, LOD is as it is defined: the concentration equal to blank sample plus three standard deviations in an experiment performed under laboratory conditions. LOD should not be considered as a real-world expectation value for deployable bioanalysis systems as it is determined under ideal and controlled laboratory conditions.

In general the sensor developer or assessor designs the experiments so that LOD and LOQ are primarily properties of the sensor and not significantly dependent on assay procedure or receptor-ligand performance. If the sponsor intends to do further technology advancement or undertake procurement, it is critical that the focus of an evaluation is on the sensor system and not on the assay. Thus the molecular recognition element, the analyte and the wetware should be designed to be as simple and robust as possible in order to focus the experiment on the sensor performance. For biosensor development the nucleic acid hybridization described above is a useful model. Also useful as a molecular recognition pair are streptavidin and biotin-labeled molecules [1]. These can be purchased as purified ready-to-use commercial products. Until a biosensor system has been well tested using robust receptor-ligand pairs it is advisable to avoid using deactivated agents or deactivated pathogen-like materials. Even though there might be no safety concern with such agent simulants, the materials generally are not single molecular compounds and the LOD results cannot be defined in molecular concentrations. For streptavidin-biotin the low dissociation constant ($K_D \approx 10^{-14}$ M) is indicative of a very strong binding interaction. Although streptavidin-biotin and dA_{20}-dT_{20} are not real-world detection targets, they provide the developer or assessor with ready access high quality reagents to determine performance characteristics. Additional reliable receptor-ligand (antibody-antigen)

pairs are available from commercial sources [1]. Comparisons of biosensor performance with other detection systems or with work carried out other laboratories are made easier by employing readily available commercial reagents. With commercial reagents there are fewer logistic problems with sourcing reagents in order to replicate or compare to work of others.

3.5.2 Specificity and selectivity

The terms specificity and selectivity have different meanings for analytical chemistry and receptor-ligand binding assays [21]; here we use terminology for receptor-ligand binding. Specificity in a receptor-ligand assay refers to the degree of discrimination between target analyte and nontarget (negative) samples or between analyte and a structurally similar molecule. Specificity is the relative affinity of sensor to the target versus a non-target. In mathematical terms specificity can be expressed the inverse ratio of dissociation constants, i.e.,

Specificity (target versus nontarget) = $(1 / K_{D\ target}) \div (1 / K_{D\ nontarget})$

or = $K_{D\ nontarget} / K_{D\ target}$

Specificity can be considered as the complement of cross-reactivity: greater specificity is less cross-reactivity [15, 22]. Specificity will be primarily an issue associated with the molecular recognition system and not highly dependent upon hardware.

Selectivity is the ability of the sensor to detect the particular analyte in mixtures of interfering substances or sample matrices. Selectivity can be characterized in several ways. It can be expressed as the ratio of the signal from analyte alone to the signal of the interfering substance at the same concentration as the analyte, i.e., a comparison of calibration curves of the analyte and interfering substance run independently. Another method to assess selectivity is to spike the interferent at an expected concentration into the analyte sample(s) and compare the output signal of the sensor of spiked to non-spiked, i.e., the ratio of spiked to non-spiked. The latter method is useful for assessing selectivity in various matrices [22]. Selectivity is a less quantifiable characteristic than specificity. Usually specificity or selectivity issues can be corrected by use of an alternative MRE or by pre-assay sample processing. The issues are rarely the fault of the underlying transducer/sensor technology. The problem primarily lies with the assay itself and can be effectively resolved by improving the assay protocol. However in some situations, extreme sample matrix effects can overcome the ability of the sensor to return reliable results. Problems can be observed in blood plasma or serum samples and in environmental samples containing soils. The effects are attributable to lipids, proteins, carbohydrates and metabolites in high concentration (blood products), or to metal ions, resins, and pH (soils, etc.). In most cases these matrix effects can be remedied by 3–10 fold dilution since the transducer component of the sensor is not permanently impaired by the interferents. Samples containing highly corrosive or resinous material could degrade the transducer rendering it inoperative, although the likelihood is low. Such cases would be ameliorated with sample dilution (in buffer). It is good practice at the early development stage to determine the susceptibility of the transducer/sensor to potentially corrosive materials.

3.5.3 Other characteristics and evaluation criteria

Two analytical properties that arise from an assay calibration curve are accuracy and precision. Accuracy refers to how well the returned value of a test sample compares to the true concentration. Precision refers to how replicate test samples compare to each other. A notional assay could return three replicate samples with standard deviation within 1% of the mean which would indicate good precision. However the mean could be 150% of the true value indicating less than ideal accuracy. Conversely, an assay could be more accurate but less precise. Each of these cases would provide the developer with insight about the complete test system.

The present report is directed at sensors at lower Technology Readiness Levels (TRL) [23] and as such the performance characteristics discussed are basic and pertain to the sensor itself. As a technology progresses towards commercial and field readiness other properties and assessment criteria would be applied. Table 1 shows an evaluation scheme that was developed by US Army Edgewood for commercial-of-the-shelf (COTS) biosensor technologies [24]. The Edgewood Test Bed (2014) [19] has been analyzed and reviewed by DRDC for potential application to technology evaluations within CAF acquisitions [20]. The principles developed within the Test Bed are relevant to biodetection technologies at all TRLs.

Table 1: *Distribution of scenario evaluation weightings.* *[1]

Evaluation criterion	Field use	Mobile laboratory /field laboratory	Diagnostic use and point-of-care laboratory	High-sensitivity, high-throughput analytical laboratory
Throughput of product	0.03	0.05	**0.11**	**0.20**
Re-use	0.06	0.03	0.02	0.02
Sensitivity and detection	0.03	**0.12**	**0.24**	**0.27**
Signature	0.06	0.04	0.03	0.01
Training	0.01	0.01	0.01	0.01
Speed	0.06	0.06	0.06	0.06
Transportability	**0.24**	**0.12**	0.01	0.01
Physical systems requirements	**0.16**	**0.12**	0.08	0.03
System maturity	0.01	0.01	0.01	0.01
Operational conditions	**0.14**	0.10	0.05	0.01
Maintenance	0.06	0.06	0.05	0.03
Ease of use	0.07	0.06	0.06	0.05
Interoperability and system complexity	0.06	0.05	0.04	0.03
Versatility of sample input	0.01	**0.17**	**0.23**	**0.26**
Evaluation total scores	1.00	1.00	1.00	1.00

*Reproduced from Ref 20; adapted from Reference 24.
[1]The weightings represent the relative importance of each criterion to the particular scenario of use. The highly weighed criteria are in bold face.

4 Conclusions and comments

The biosensor comprises a transducer and a molecular recognition element (receptor). Both are required to function in order to generate an output signal. Also required are wetware reagents and high quality target analyte (ligand). The biosensor performance is dependent on all the individual inputs. In the initial stages of development the focus is on the transducer hardware device and thus the focus of the evaluation should be on the transducer. The challenge for the biosensor developer is to construct test experiments directed at the underlying problem of transducer/biosensor technology. In describing such a problem and reporting results, the terms "transducer" and "biosensor" might sometimes inadvertently be interchanged since they are intimately employed together and function together. Despite difficulty in terminology, the focus of such work and of this report is on these underlying components. The goal of the test experiments and the assessment is to extract the inherent characteristics of the sensor, such as the dynamic range of signal, sensitivity, limit of detection and stability. Thus simple, easy-to-use, commercially available molecular recognition elements and target analytes are advantageous. Any urge to test the sensor system with real-world simulants or deactivated agents should be resisted until simple receptor-ligand pairs have been tested and the underlying utility of the technology has been demonstrated.

This report is an outline for assessing the performance characteristics of biosensor technology under development. In the context of the nanowire biosensor, the first assessment would be to confirm that the transducer will function in contact with aqueous solutions. If the proposed device incorporates molecular recognition elements (i.e., receptors) on the surface, then a simple and robust receptor-ligand pair should be employed to demonstrate that the device functions. For the most part, the response of the sensor is dependent receptor-ligand interaction which in turn is dependent on the bulk properties of the ligand, usually a macromolecule such as protein, nucleic acid or polysaccharide. In the present example of a nanowire FET biosensor, the receptor-bound ligand affects the charge density on the transducer surface which in turn modulates the electron conductance in the nanowire (i.e., the modulation of conductance is the signal). For the EIS sensor (Figure 5), the same receptor-bound ligand molecule affects the electron charge-transfer resistance through the sensor surface (i.e., the modulation of charge-transfer resistance is the signal). Each sensor device is sensitive to the macromolecules on the surface in its own way.

A biosensor based on receptor-ligand interactions should display the characteristics of receptor-ligand binding as presented herein. A plot of signal versus ligand concentration should resemble the equilibrium binding curves shown above (Figures 3b and 5). This simple concept is often overlooked by researchers. In general, there is no expectation that all sensor experiments will return the same results but there is the expectation to observe basic characteristics of receptor-ligand binding. A benefit of using simple receptor-ligand pairs is that the experimental data is more comparable to published work. However, in practice each published work contains many system unknowns such as receptor coverage on the transducer, background noise, transducer response, conjugation chemistry, etc. Thus extensive comparisons are problematic.

As indicated above, the experimental exercise is to assess the transducer not the wetware, so simple wetware simplifies the problem. Determination of limits of detection, signal-to-noise ratio, sensitivity, stability and reproducibility will allow assessment of the transducer. If problems arise during test experiments, they are easier to troubleshoot with simple receptor-ligand pairs. Often

times the developer (or sponsor) of technology will be end-user oriented (rather than a biosensor expert). In such cases, this report will assist (end-user oriented) developers in assessing 3rd-party work and in guiding liaison with contractors. The goal of this report is to provide understanding of the underlying issues of biosensor performance. This understanding can be strategically applied to the project in order to increase the likelihood of success in current and future development.

References

[1] Rowsell SJ, Lee WE. 2016. Model analyte systems to assess nanowire biosensors. DRDC Scientific Letter, DRDC-RDDC-2016-L430, Defence Research and Development Canada.

[2] Hsieh K, Ferguson BS, Eisenstein M, Plaxco KW, Soh HT. 2015. Integrated electrochemical microsystems for genetic detection of pathogens at the point of care. *Accounts of Chemical Research*. 48, 911–920.

[3] Gayathri SB, Kamaraj P. 2015. Development of electrochemical DNA biosensors-a review. *Chemical Science Transactions*. 4, 303–311.

[4] Vigneshvar S, Sudhakumari CC, Senthilkumaran B, Prakash H. 2016. Recent advances in biosensor technology for potential applications–an overview. *Frontiers in Bioengineering and Biotechnology*. 4.

[5] Saveliev I, Blumin M, Lynall D, Ruda H. 2016. NanOwireBased (NOB) Biochemical Sensor Part 1. DRDC Contract Report, DRDC-RDDC-2016-C259; Saveliev I, Blumin M, Lynall D, Tseng A, Ruda H. 2016. NanOwireBased (NOB) Biochemical Sensor Part 2. DRDC Contract Report, DRDC-RDDC-2016-C260, Harry Ruda—Department of Materials Science and Engineering, University of Toronto.

[6] Tseng AC, Lynall D, Savelyev IG, Blumin M, Wang S, Ruda HE. 2017. Sensing responses based on transfer characteristics of InAs nanowire field-Effect transistors. *Sensors 2017*, 17, 1640; doi:10.3390/s17071640. http://dx.doi.org/10.3390/s17071640 (Access date: December 20, 2017).

[7] Mahmoud AM, Tang T, Harrison DJ, Lee WE, Jemere AB. 2014. A regenerating self-assembled gold nanoparticle-containing electrochemical impedance sensor. *Biosensors and Bioelectronics*. 56, 328–333.

[8] Zhang Y, Heller A. 2005. Reduction of the nonspecific binding of a target antibody and of its enzyme-labeled detection probe enabling electrochemical immunoassay of an antibody through the 7 pg/mL – 100 ng/mL (40 fM – 400 pM) range. *Analytical Chemistry*. 77, 7758–7762.

[9] G-Biosciences. Uncovering the Role of Detergents in Protein Solubilization. https://info.gbiosciences.com/blog/bid/172097/uncovering-the-role-of-detergents-in-protein-solubilization (Access date: December 20, 2017).

[10] Caligur V. Detergents and Solubilization Reagents SigmaAldrich. *Biofiles* vol3 #3. http://www.sigma-aldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/2/biofiles_issue3_3.pdf (Access date: December 20, 2017).

[11] de Mol NJ, Fischer MJ. 2008. Kinetic and thermodynamic analysis of ligand-receptor interactions: SPR applications in drug development. *Handbook of Surface Plasmon Resonance*. pp. 123–172.

[12] Sanders CR. 2010. Biomolecular ligand-receptor binding studies: theory, practice, and analysis. Vanderbilt University Nashville.

[13] Zhu Z, Feng M, Zuo L, Zhu Z, Wang F, Chen L, Li J, Shan G, Luo SZ. 2015. An aptamer based surface plasmon resonance biosensor for the detection of ochratoxin A in wine and peanut oil. *Biosensors and Bioelectronics*. 65, 320–326.

[14] Esteban Fernandez de Avila B, Watkins HM, Pingarrón JM, Plaxco KW, Palleschi G, Ricci F. 2013. Determinants of the detection limit and specificity of surface-based biosensors. *Analytical Chemistry*. 85, 6593–6597.

[15] Tijssen P. 1985. Practice and theory of enzyme immunoassays. Elsevier.

[16] Sanagi MM, Ling SL, Nasir Z, Hermawan D, Wan Ibrahim WA, Naim AA. 2009. Comparison of signal-to-noise, blank determination, and linear regression methods. *Journal of AOAC International*. 92, 1833–1838.

[17] Long GL, Winefordner JD. 1983. Limit of detection. A closer look at the IUPAC definition. *Analytical Chemistry*. 55, 712A–724A.

[18] Detection limit – Wikipedia. https://en.wikipedia.org/wiki/Detection_limit (Access date: December 20, 2017).

[19] Betters J, Goldburn C, Sabol C, Dorsey R, Emanuel P. 2013. Edgewood test bed hand-held and man-portable edition. Army Edgewood Chemical Biological Center APG MD, Research and Technology Dir.

[20] Lee W. 2016. Review and analysis of bioidentification systems for mobile laboratory and field use. DRDC Scientific Report, DRDC-RDDC-2016-R193, Defence Research and Development Canada.

[21] Danzer K. 2001. Selectivity and specificity in analytical chemistry. General considerations and attempt of a definition and quantification. *Fresenius Journal of Analytical Chemistry*. 369, 397–402.

[22] Thévenot DR, Toth K, Durst RA, Wilson GS. 2001. Electrochemical biosensors: recommended definitions and classification. *Biosensors and Bioelectronics*. 16, 121–131.

[23] Technology Readiness Levels in the Department of Defense (DoD) (Source: DoD 2010 Defense Acquisition Guidebook) https://www.army.mil/e2/c/downloads/404585.pdf (Access date: December 20, 2017).

[24] Emanuel P, Caples M. Chemical, biological, radiological technology survey, 2011. US Army Edgewood Chemical Biological Center, APG MD.

List of symbols/abbreviations/acronyms/initialisms

BioDIM	Biological detection, identification, and monitoring
CAF	Canadian Armed Forces
COTS	Commercial-of-the-shelf
dA_{20}	Polydeoxyadenine
dT_{20}	Polydeoxythymine
D CBRN D	Directorate Chemical Biological Radiological Nuclear Defence
DND	Department of National Defence
DRDC	Defence Research and Development Canada
EIS	Electrochemical impedance spectroscopy
LOD	Limit of detection
LOQ	Limit of quantification
MRE	Molecular recognition elements
NSB	Nonspecific binding
NWFET	Nanowire field effect transistor
R&D	Research & Development
S/N	Signal-to-noise ratio
TRL	Technology Readiness Level

DOCUMENT CONTROL DATA

(Security markings for the title, abstract and indexing annotation must be entered when the document is Classified or Designated)

1. **ORIGINATOR** (The name and address of the organization preparing the document. Organizations for whom the document was prepared, e.g., Centre sponsoring a contractor's report, or tasking agency, are entered in Section 8.)

DRDC – Suffield Research Centre
Defence Research and Development Canada
P.O. Box 4000, Station Main
Medicine Hat, Alberta T1A 8K6
Canada

2a. **SECURITY MARKING** (Overall security marking of the document including special supplemental markings if applicable.)

CAN UNCLASSIFIED

2b. **CONTROLLED GOODS**

(NON-CONTROLLED GOODS)
DMC A

3. **TITLE** (The complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title.)

Performance characteristics to assess a receptor-ligand biosensor

4. **AUTHORS** (last name, followed by initials – ranks, titles, etc., not to be used)

Lee, W.E.; Wang, S.; Rowsell, S.J.

5. **DATE OF PUBLICATION** (Month and year of publication of document.)

March 2018

6a. **NO. OF PAGES** (Total containing information, including Annexes, Appendices, etc.)

30

6b. **NO. OF REFS** (Total cited in document.)

24

7. **DESCRIPTIVE NOTES** (The category of the document, e.g., technical report, technical note or memorandum. If appropriate, enter the type of report, e.g., interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Scientific Report

8. SPONSORING ACTIVITY (The name of the department project office or laboratory sponsoring the research and development – include address.)

DRDC – Suffield Research Centre
Defence Research and Development Canada
P.O. Box 4000, Station Main
Medicine Hat, Alberta T1A 8K6
Canada

9a. **PROJECT OR GRANT NO.** (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)

9b. **CONTRACT NO.** (If appropriate, the applicable number under which the document was written.)

10a. ORIGINATOR'S DOCUMENT NUMBER (The official document number by which the document is identified by the originating activity. This number must be unique to this document.)

DRDC-RDDC-2017-R178

10b. OTHER DOCUMENT NO(s). (Any other numbers which may be assigned this document either by the originator or by the sponsor.)

11. DOCUMENT AVAILABILITY (Any limitations on further dissemination of the document, other than those imposed by security classification.)

Unlimited

12. DOCUMENT ANNOUNCEMENT (Any limitation to the bibliographic announcement of this document. This will normally correspond to the Document Availability (11). However, where further distribution (beyond the audience specified in (11) is possible, a wider announcement audience may be selected.))

Unlimited

13. **ABSTRACT** (A brief and factual summary of the document. It may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall begin with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (S), (C), (R), or (U). It is not necessary to include here abstracts in both official languages unless the text is bilingual.)

Biosensors represent an important area of technology for biodetection, bioidentification, diagnostics, medical countermeasures, and human performance within the operations of the Canadian Armed Forces (CAF). The development of biological detection, identification, and monitoring (BioDIM) systems is a multi-step process that requires the assessment of candidate biosensor technologies at each step for further technology advancement. The CAF Directorate Chemical Biological Radiological Nuclear Defence (D CBRN D) sponsors research and development of biosensor technology within the DRDC CBRN Defence Program Inform Project. The present report contains an overview of the fundamental concepts associated with the predominant class of biosensors, those based on receptor-ligand interactions. Such biosensors comprise two distinct components, an electronic transducer device and molecular recognition elements (MREs) that together are able to detect particular chemical or biological analyte molecules. In a biosensor technology development project, normally the sponsor directs resources at the electronic transducer component plus hardware of the BioDIM system rather than the MREs. The focus of the report is on the performance characteristics of the transducer plus associated hardware, i.e., the areas where the sponsor would direct resources. This report outlines some performance characteristics of biosensors and provides analysis and guidance for assessing new biosensor technologies. The objective of the report is to provide CAF sponsors with tools and strategies for assessing biosensor development, hence to enhance the return on investment of DND resources.

Les biocapteurs représentent un domaine important de la technologie pour la biodétection, la bioidentification, le diagnostic, les contre-mesures médicales et le rendement humain dans le cadre des opérations des Forces armées canadiennes (FAC). La mise au point de systèmes de biodétection, bioidentification et biosurveillance (BioDIM) est un processus qui comporte de multiples étapes et qui nécessite l'évaluation des technologies de biocapteurs susceptibles d'être utilisées à chacune de ces étapes pour l'avancement technologique. La Direction – Défense chimique, biologique, radiologique et nucléaire, (DDCBRN) des FAC parraine la recherche et le développement de la technologie des biocapteurs au sein du projet INFORM dans le cadre du Programme de défense CBRN de RDDC. Le présent rapport contient un aperçu des concepts fondamentaux relatifs à la classe prédominante de biocapteurs, soit ceux basés sur les interactions récepteurs-ligands. Ces biocapteurs comportent deux composants distincts, un transducteur électronique et des éléments de reconnaissance moléculaire (ERM) qui, ensemble, permettent de détecter certaines molécules chimiques ou biologiques à analyser. Dans un projet de développement technologique de biocapteurs, le parrain affecte normalement les ressources au composant du transducteur électronique et au matériel du système de BioDIM, plutôt qu'aux ERM. Le présent rapport porte principalement sur les caractéristiques de rendement du transducteur et du matériel connexe, c.-à-d. les secteurs auxquels le parrain affecte normalement les ressources. Ce rapport présente les caractéristiques de rendement des biocapteurs et fournit une analyse, de même qu'un encadrement afin d'évaluer de nouvelles technologies de biocapteurs. L'objectif du rapport est de doter les parrains des FAC d'outils et de stratégies pour évaluer la mise au point des biocapteurs, et améliorer ainsi le rendement du capital investi dans les ressources du MDN.

14. **KEYWORDS, DESCRIPTORS or IDENTIFIERS** (Technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g., Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

Performance characteristics; receptor-ligand interactions; biosensors